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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DIPHENHYDRAMINE AND NAPROXEN BY USING RP-HPLC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The main aim of present analytical research is to develop a simple, accurate, rapid and economical RP-HPLC method for the simultaneous estimation of Diphenhydramine and Naproxen in bulk and pharmaceutical dosage forms . The drug analysis plays an important role in the development, manufacture and therapeutic use of drug. To validate the method according to ICH guidelines.

KEYWORDS: Diphenhydramine, Naproxen, Methanol, Buffer.

Plan of work: An attempt was made in an stepwise manner to develop a simple, rapid, selective method using high performance liquid chromatography (RP) for Diphenhydramine and Naproxen. The following stepwise manner protocol was followed.

- An attempt was made in a step wise manner to develop a simple, rapid, selective method using high performance liquid chromatography (RP) for Diphenhydramine and Naproxen combination.
- The following stepwise protocol was followed.
- As a startup, literature survey has done for the parameters like solubility chemical structure, pka value and analytical profile.
- From the data obtained by literature & practically, UV spectroscopic method has been chosen for the detection of λmax using the selective solvent and validated.
- Later several trails were done in RP-HPLC using a different combinations of mobile with available columns to optimize the method.

- After optimization of HPLC method validation of analytical method was done as per ICH guidelines.

Literature Review

R.D. Toothaker et al., The potential for a pharmacokinetic interaction between naproxen and diphenhydramine was examined in a randomized three-way crossover design with a 1-week washout between dosing. Single oral doses of 220 mg of naproxen sodium and 50 mg of diphenhydramine hydrochloride were given separately and together to 30 healthy male and female subjects. Heparinized blood samples obtained for 48 h postdose were assayed for plasma naproxen and diphenhydramine concentrations using validated high-performance liquid chromatography (HPLC) and gas chromatography (GC) assay methods, respectively. The area under the plasma concentration–time curve (AUC), maximum plasma concentrations (*C*max), time of *C*max (*T*max) and terminal exponential half-life ($t_{1/2,z}$), were analysed for significant treatment differences by analysis of variance (ANOVA). Based on absence of significant treatment effects on AUC and *C*max, single-dose oral co-administration of 220 mg of naproxen sodium with 50 mg of diphenhydramine hydrochloride does not alter the pharmacokinetics of either naproxen or diphenhydramine. Significant treatment differences seen for naproxen *T*max (0.3 h, males only) and diphenhydramine $t_{1/2,z}$ (0.8 h, females only) were minor and are unlikely to have therapeutic consequences. Thus, efficacy and safety of concomitant naproxen and diphenhydramine should not be altered due to a pharmacokinetic interaction. Copyright © 2000 John Wiley & Sons, Ltd.32.

Jayaraman Anbu et al., Mesalamine is a potent ‘‘reverse transcriptase inhibitor ’’ belonging to the class of nucleoside analog reverse transcriptase inhibitor (NARTI). Olanzapine is an anti-HIV treatment drug in the class of drugs called nucleoside reverse transcriptase inhibitors (NRTIs). Both are excellent anti-retroviral drugs, widely used clinically in the treatment of infections which are related to immuno deficiency virus. The present combination of Mesalamine and Olanzapine was marketed as one tablet dosage form formulation with a dose of Mesalamine 150mg/tab and Olanzapine 40mg/tab. Although various methods have been developed for the estimation of Mesalamine and Olanzapine individually and in combination with other drugs, no official method has been published with the combination of these drugs. The fixed dose combination of Mesalamine and Olanzapine was subjected to simultaneous estimation by Reverse phase HPLC method.

Mokhtar M. Mabrouk et al., Highly sensitive, simple and accurate reversed phase liquid chromatographic and first derivative spectrophotometric methods were developed for determination of the non-steroidal anti-inflammatory drugnaproxen and the antihistaminic diphenhydramine in their binary mixtures. The HPLC method involves separation of naproxen and diphenhydramine on XBridgeTMC18 reversed phase (4.6×150 mm, particle size 5µm) column using a mobile phase consists of ethanol: phosphate buffer; pH 3in a ratio of 60:40 (v/v). The flow rate was 1 mL.min-1with ultraviolet detection at 216 nm.

MATERIALS AND METHODS

Instruments

- HPLC –Waters Model NO.2690/5 series Compact System Consisting of Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS).
- Sonicator(FAST CLEAN).

Chemicals

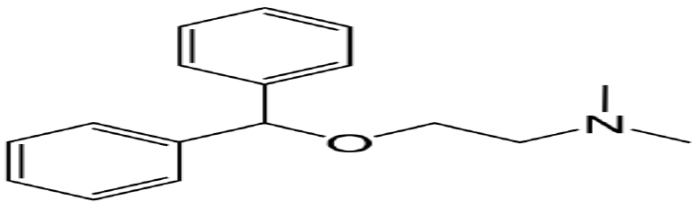
- Methanol HPLC Grade.
- Buffer (KH2PO4) HPLC Grade.

Raw Material

Metformin and Alogliptin Working Standards.

Drug Profile

Diphenhydramine



Chemical Formula : C17H21NO

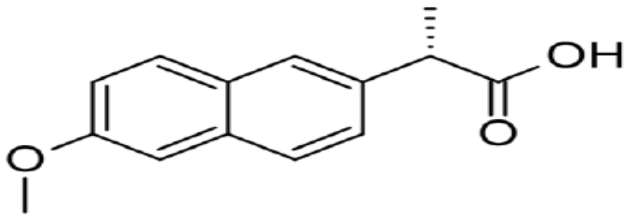
Molecular Weight : 255.354 g/mol

IUPAC name : 2 -(diphenylmethoxy)-N,N-dimethylethanamine

Description : Diphenhydramine competes with free histamine for binding at HA-receptor sites. This antagonizes the effects of histamine on HA-receptors, leading to a reduction of the negative symptoms brought on by histamine HA-receptor binding.

Category : Anti-histamine drug

Alogliptin



Chemical formula **:** C 14H14O3

Molecular Weight **:** 230.25 g/mol

IUPAC name **:** (2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid

Description : The mechanism of action of naproxen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity.

Category : NSAID drug

Method Development Trials

Trial-1: Chromatographic conditions

Column : C18Symmetry

Flow rate : 1ml/min

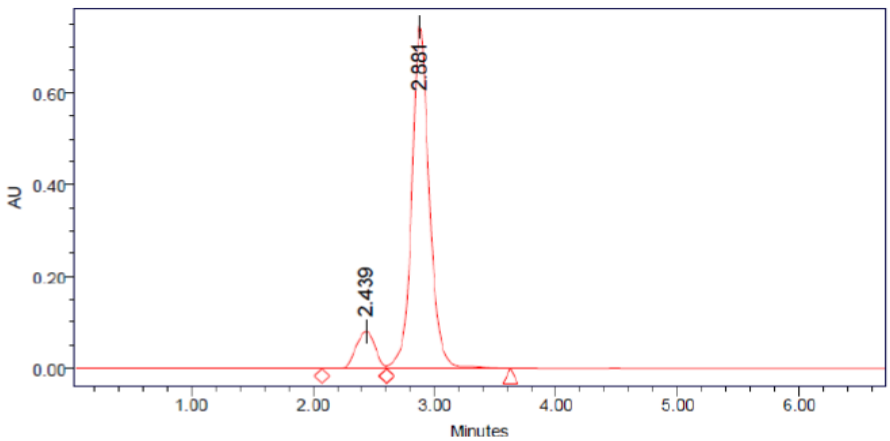
Wavelength : 254 nm

Mobile phase : Methanol: Phosphate buffer PH3 (70:30)

Injection volume : 10 µL

Column Temperature :Ambient

Chromatogram of Trial 1.



Observation: There was no proper peak separation

Trial. 2: Chromatographic conditions.

Column : Symmetry C18 (4.6 x 150mm, 5□m, Make: Waters)

Flow rate : **0.8**ml per min

Wavelength : 254 nm

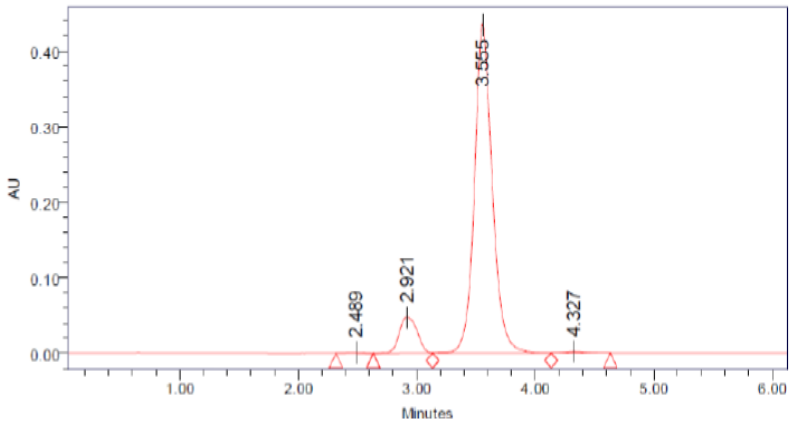
Mobile phase : Methanol: Sodium acetate PH 4 (60:40)

Injection volume : 10 µL

Column Temperature : Ambient

Retention time :10 min

Chromatogram of Trial 2.



Observation: Peaks are separated but tailing has been observed.

Trial. 3: Chromatographic conditions.

Column : Symmetry C18 (4.6 x 150mm, 5□m, Make: Waters)

Flow rate : **2** ml /min

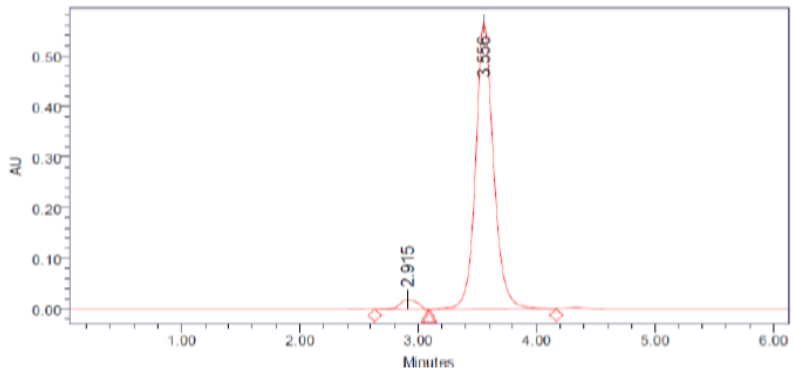
Wavelength : 254 nm

Mobile phase : Methanol: Ammonium acetate PH3 (70:30)

.Injection volume : 10 µL

Column Temperature : Ambient

Chromatogram of Trial 3.



Retentiontime : 10min

Observation : Peaks were eluted but with less resolution peaks were seen

Optimized Chromatographic Conditions

Preparation of mobile phase: Take 6.8 gm of KH2PO4 into 1000ml volumetric flask dissolved in HPLC graded water and adjust pH upto 3 with ortho phosphoric acid. From the above prepared buffer take 300 ml(30%)and 700ml Methanol(70%) HPLC were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard stock solution: 75 mg of Diphenhydramine and 10mg of Naproxen were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added and sonicate to dissolve it completely. The volume was made up to the mark with the same solvent. (Stock solution) .Further 0.1 ml was pipette out from the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent to give the concentration of 750 µg/ml and 100 µg/ml respectively.

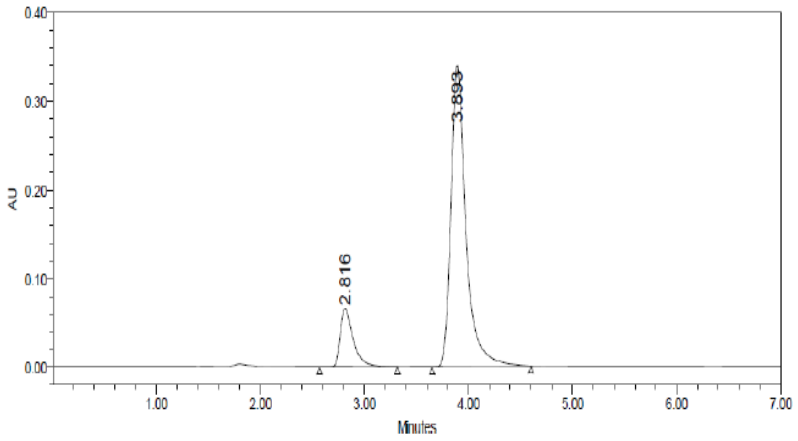
Preparation of sample solution: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg of Diphenhydramine and 10mg of Naproxen was weighed and dissolved in 80 ml of methanol. Then it is sonicated for 30 min and solution was filtered through 0.45 µ membrane filter into a 100 ml volumetric flask. Filter paper was washed with the solvent, adding washings to the volumetric flask and volume was made up to mark. Further working sample solutions of 100µg/ml and 10µg/ml of Diphenhydramine and Naproxen was prepared with mobile phase respectively.

Optimized chromatographic conditions

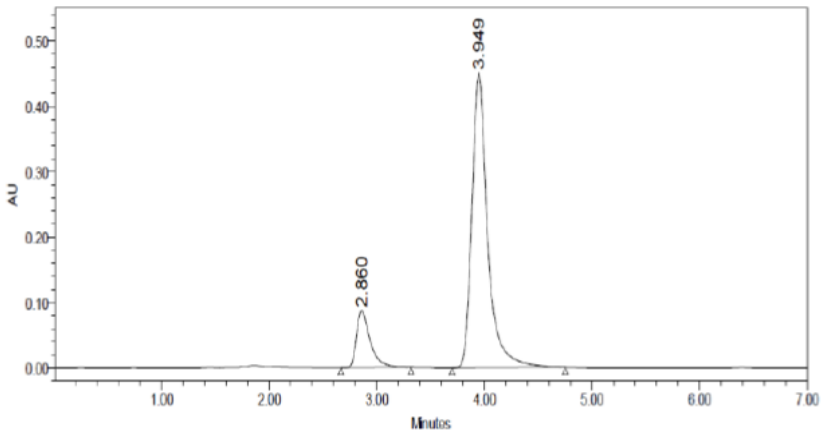
Parameters	Method
Stationary phase (column)	Symmetry C18
Mobile Phase	Methanol and Buffer in the ratio of 80:20 V/V.
Flow rate (ml/min)	0.8 ml per min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	10
Detection wavelength (nm)	254 nm
Drug RT (min)	10 min

Observation: Resolution between two analytes was good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found to be within the acceptance criteria. Hence the method was considered to be optimized.

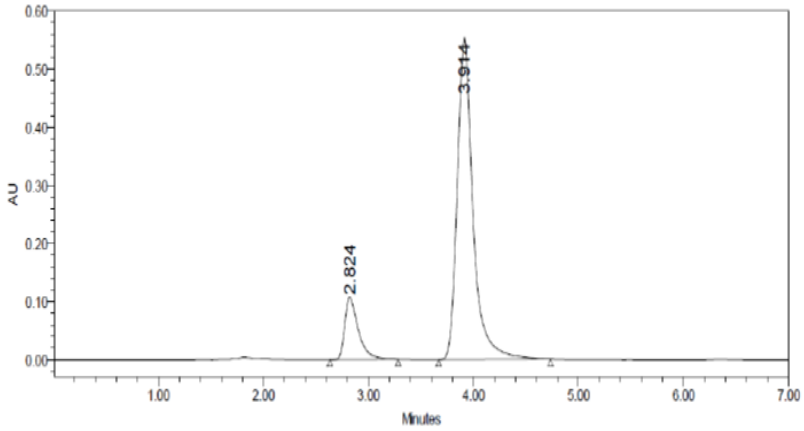
Accuracy: Accuracy at 50%



Accuracy at 100%



Accuracy at 150%



Precision

System Precision

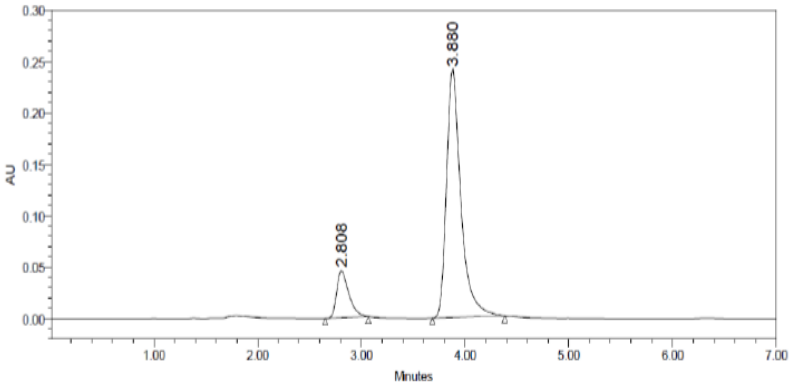


Fig. 5: Chromatogram for System Precision.

Method Precision

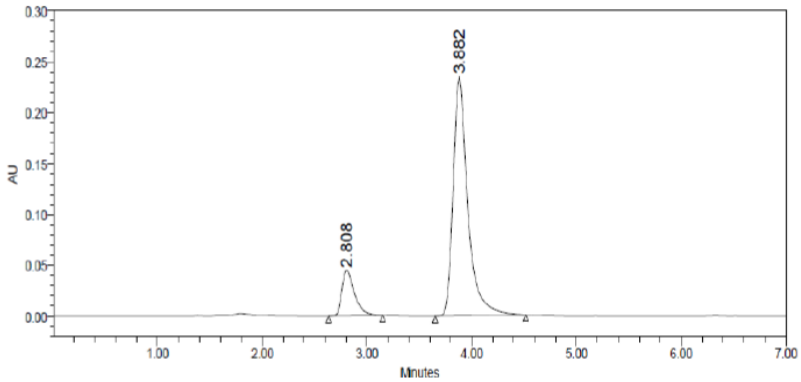
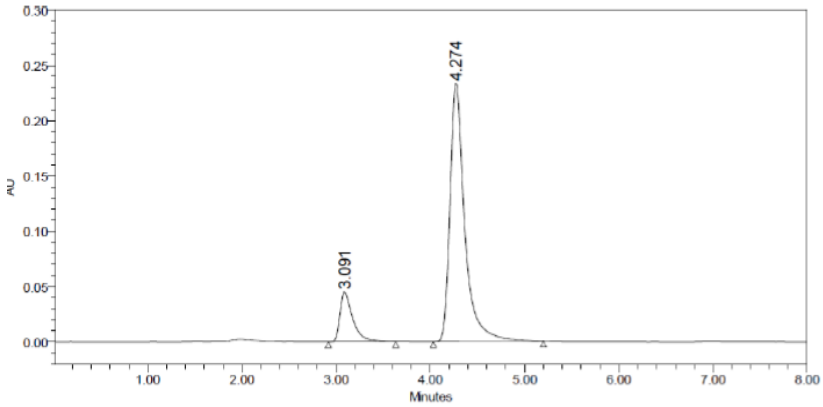


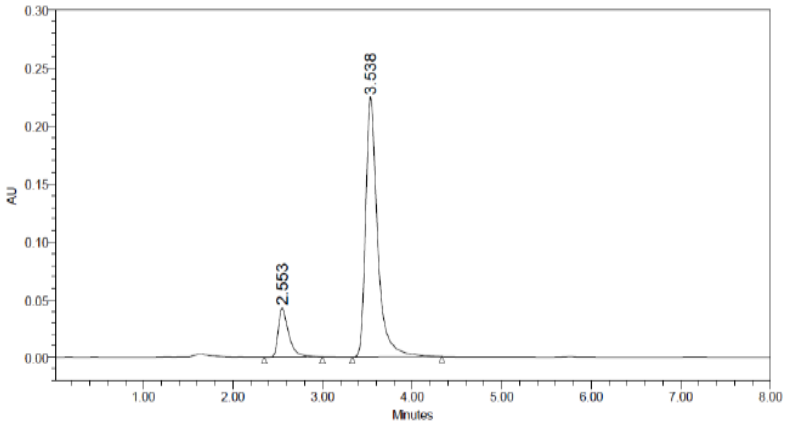
Fig. 6: Chromatogram for Method Precision.

Robustness

With Flow Rate

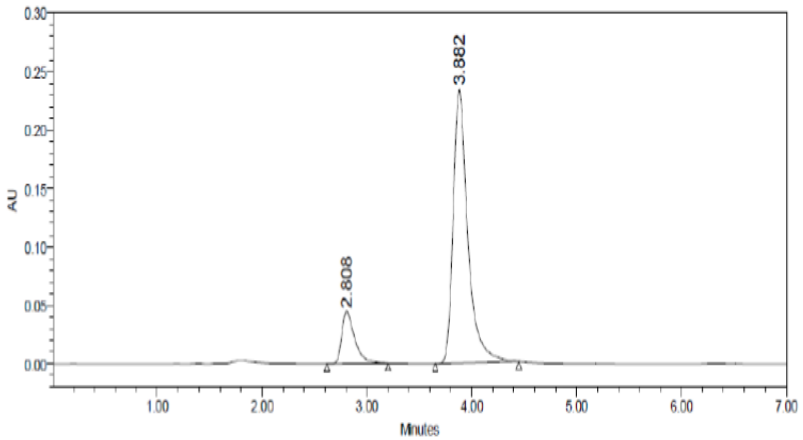


Robustnesstudies for Less flow.



Robustnes studies for More flow

Ruggedness



Ruggedness of Diphenhydramine and Naproxen

Validation Parameters

Table. 2: Validation Studies for Diphenhydramine & Naproxen.

S. No	Parameter	Results for Diphenhydramine	Results for Naproxen
1	Precision	% RSD= 0.23793	% RSD= 0.5232
2	Method precision	% RSD= 0.9	% RSD= 0.7
3	Accuracy	%Recovery= 98.0 to 102.0%	% Recovery= 98.0 to 102.0%.
4	Linearity	R2= 0.99932	R2= 0.99916
5	Limit of detection	3.041µg/mL	3.08 µg/mL
6	Limit of quantification	9.79 µg/mL	10.37 µg/mL
7	Robustness	Deliberate change	Deliberate change
8	Ruggedness	% RSD= 0.2481	% RSD= 0.287
9	Specificity	Degradation was Observed	Degradation was Observed

CONCLUSION

1. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Diphenhydramine and Naproxen . LOD and LOQ were found to be within limit.

2. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear.

➢ The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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